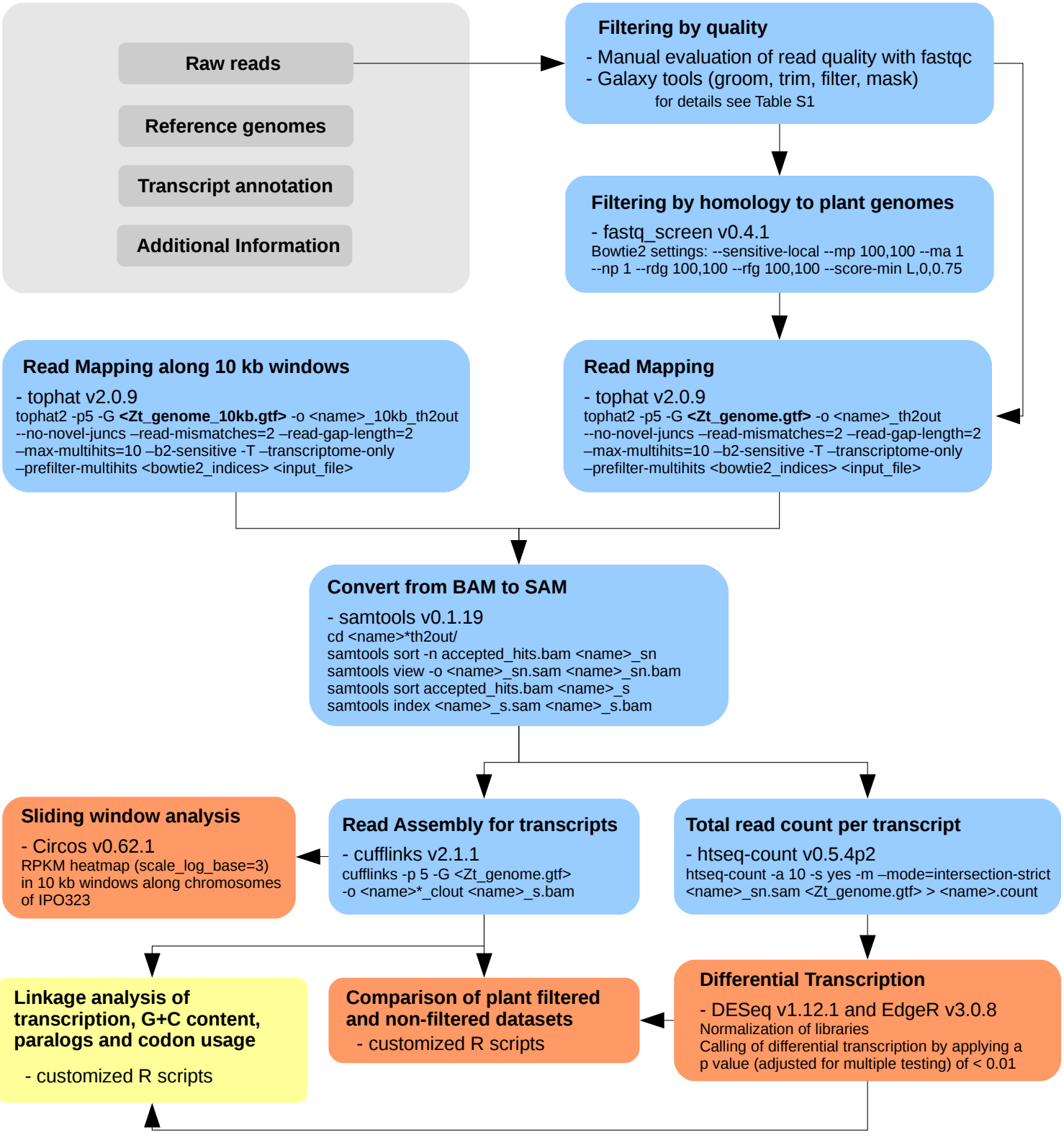
Figure S1 - Workflow and settings for RNAseq data analysis.



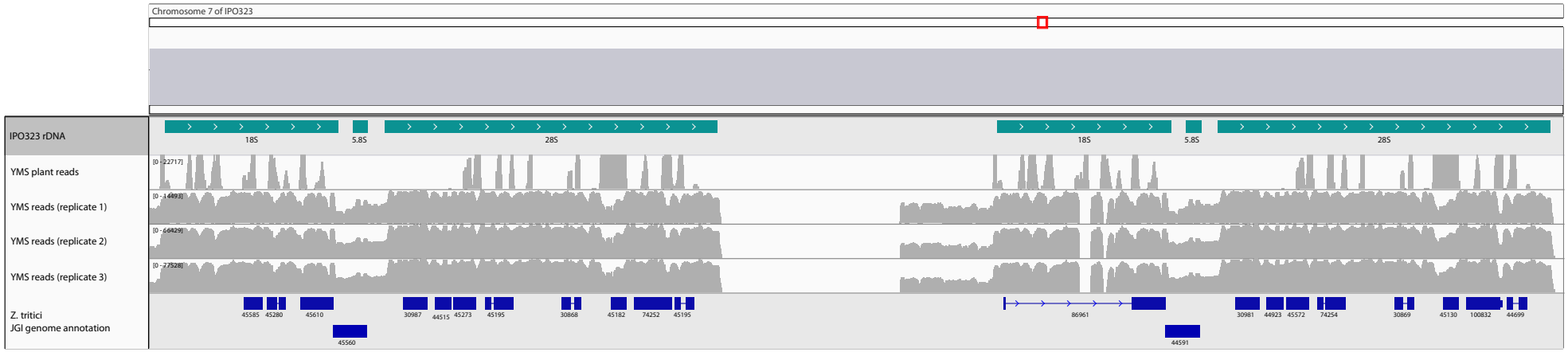


Figure S2 - Annotation of ribosomal DNA in IPO323.
Partial sequence of chromosome 7 of IPO323 encoding two tandems of 18S, 5.8S and 28S rDNA. Below the coverage of mapped RNAseq reads plotted in log-scale. The first track corresponds to a merged dataset of fungal RNAseq reads that were obtained from axenic culture of IPO323 (YMS) and that share high sequence identity to sequences in the genomes of *Triticum aestivum* (Brenchley et al. 2012) and *Brachypodium distachyon* (Bd21, The International Brachypodium Initiative 2010). Below coverage of the remaining reads is plotted for each of the three replicates from axenic culture. The range of read coverage is given in the upper left of each graph. Blue boxes below depict transcript positions and gene IDs of the JGI annotation (Goodwin et al. 2011).

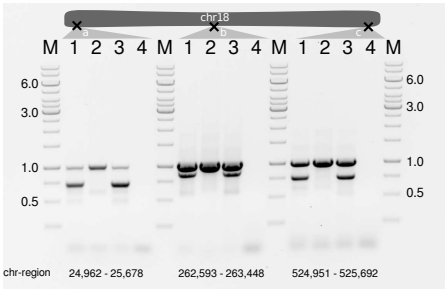


Figure S3 - Presence-absence sequence polymorphism of chromosome 18 in IPO323.
Multiplex PCR of loci at chromosome 18 and chromosome 7. The latter is used as a positive control. Chromosome-specific primers were directed against three regions of chromosome 18 (a, b, c) and one region of chromosome 7. Chromosome positions of amplified fragments are given below. M – 1kb DNA ladder (NEB, Frankfurt Germany); 1 – IPO323 (Kema and van Silfhout 1997); 2 - IPO323Δchr18; 3 - IPO323ΔKu70 (Bowler et al. 2010); 4 - water control.

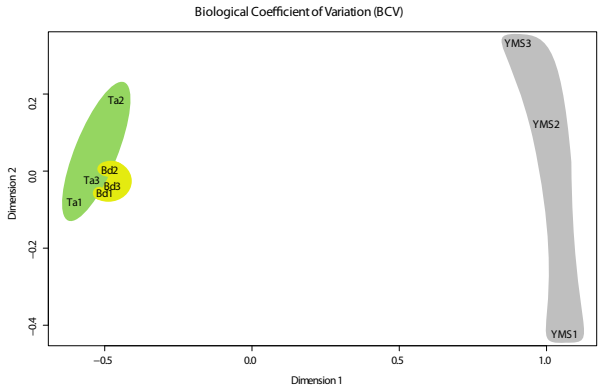
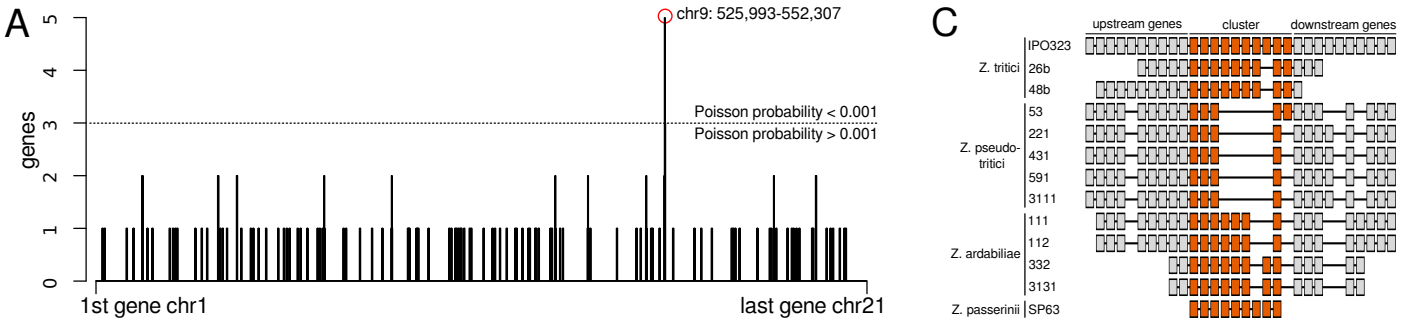


Figure S4 - Similarities between RNAseq samples based on multidimensional scaling of log-read-counts per million reads.
Plot was generated with the plotMDS function of the package edgeR (McCarthy et al. 2012) in R (www.r-project.org).
Distances between samples from axenic culture (YMS1-3) and from infected plant tissue at 4 days post inoculation of *T. aestivum* (Ta1-3) and *B. distachyon* (Bd1-3) correspond to leading biological coefficient of variation.



B

Gene ID	Chr	Length in bp	Mean RPKM of replicates Ta	Bd	YMS	Secreted	Homology
95596	9	507	0.23	0.13	0.00	no	no
75366	9	1463	1.35	1.80	0.75	no	Lysyl hydrolase glycosyltransferase family 25
24569	9	1149	1.14	0.39	1.46	no	no
47929	9	6525	1.31	3.50	212.99	no	Pleiotropic drug resistance proteins (PDR1 15) ABC superfamily
75370	9	3829	2.41	2.66	231.10	no	no
48164	9	506	38.80	57.48	440.63	no	no
95601	9	1097	10.83	11.17	0.00	no	no
17219	9	810	0.00	12.73	142.89	no	Isoamyl acetate hydrolyzing esterase and related enzymes
75376	9	867	335.20	320.76	545.88	no	Ubiquinol cytochrome c reductase subunit RIP1
28514	9	609	1.60	0.00	0.00	no	no

Figure S5 - Data are as in Figure 5 of the main text except that genes downregulated during infection of wheat and *B. distachyon* relative to axenic growth were used for analysis.

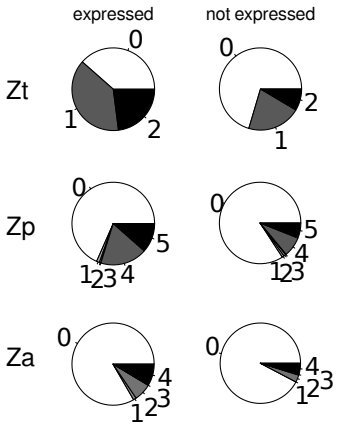


Figure S6 - Conservation of AC-encoded genes within and between *Zymoseptoria*.
Proportion of transcribed (RPKM > 2) and non-transcribed (RPKM < 2) AC-encoded genes with orthologs in the genomes of two isolates of *Z. tritici* (Zt), five isolates of *Z. pseudotritici* (Zp) and four isolates of *Z. ardabiliae* (Za). Genome sequences were obtained from Stukenbrock et al. (2011). Numbers correspond to the amount of genomes with respective proportions of orthologs.

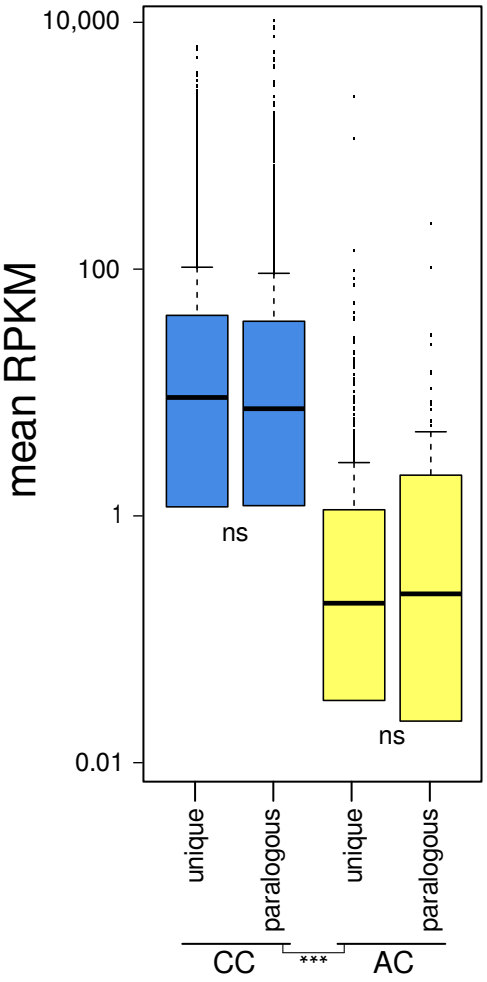
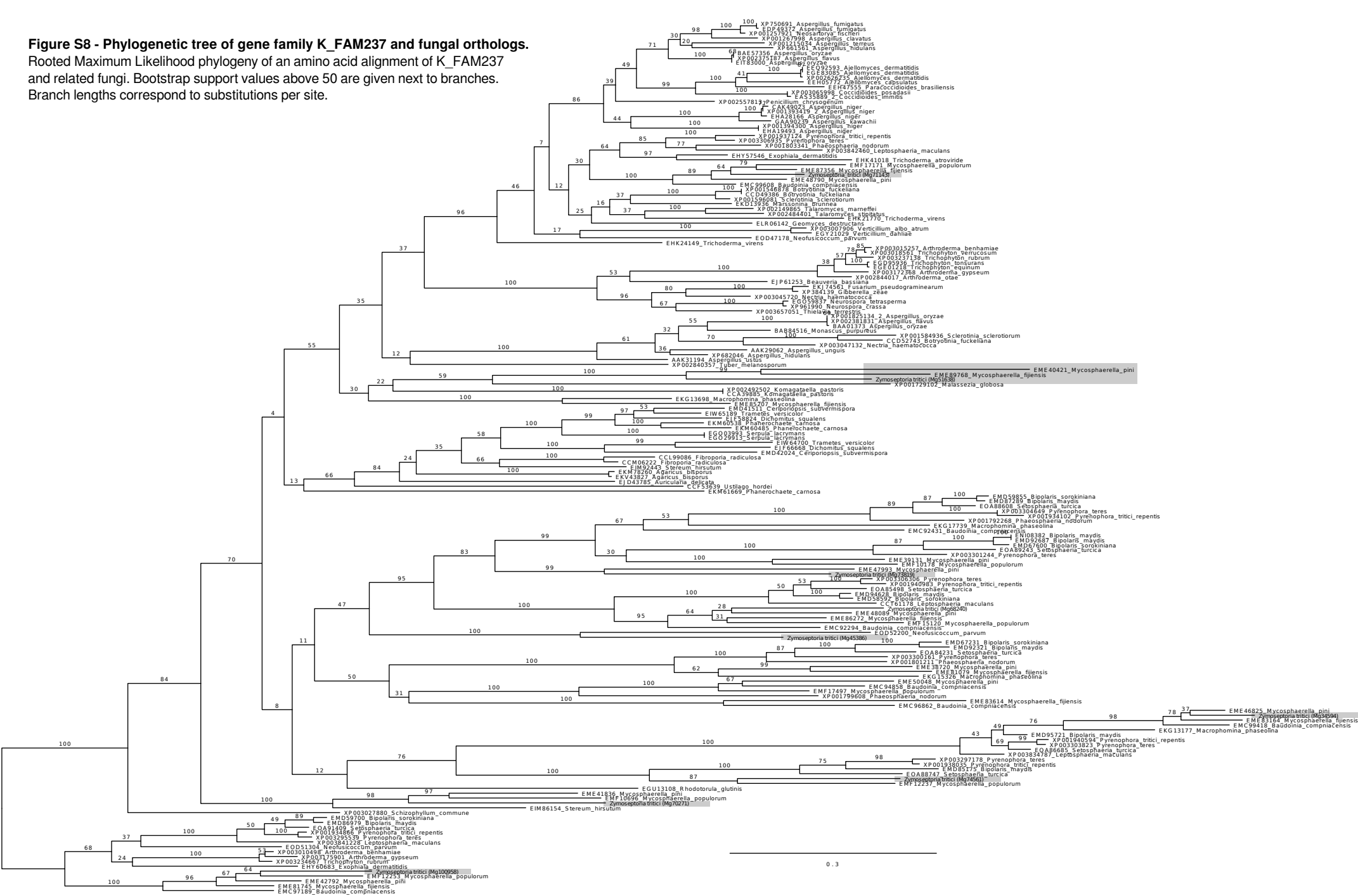


Figure S7 - RPKM comparison between unique and paralogous core and accessory chromosome-encoded genes.
Each column reports the distribution of mean RPKM values calculated from all replicates of all tested growth conditions. In a given distribution, the median is denoted as a thick horizontal bar, 25% quartiles are shown as a box, thin horizontal bars denote 1.5 times the interquartile range, and values outside the latter range are shown as points.

Figure S8 - Phylogenetic tree of gene family K_FAM237 and fungal orthologs.
Rooted Maximum Likelihood phylogeny of an amino acid alignment of K_FAM237 and related fungi. Bootstrap support values above 50 are given next to branches. Branch lengths correspond to substitutions per site.



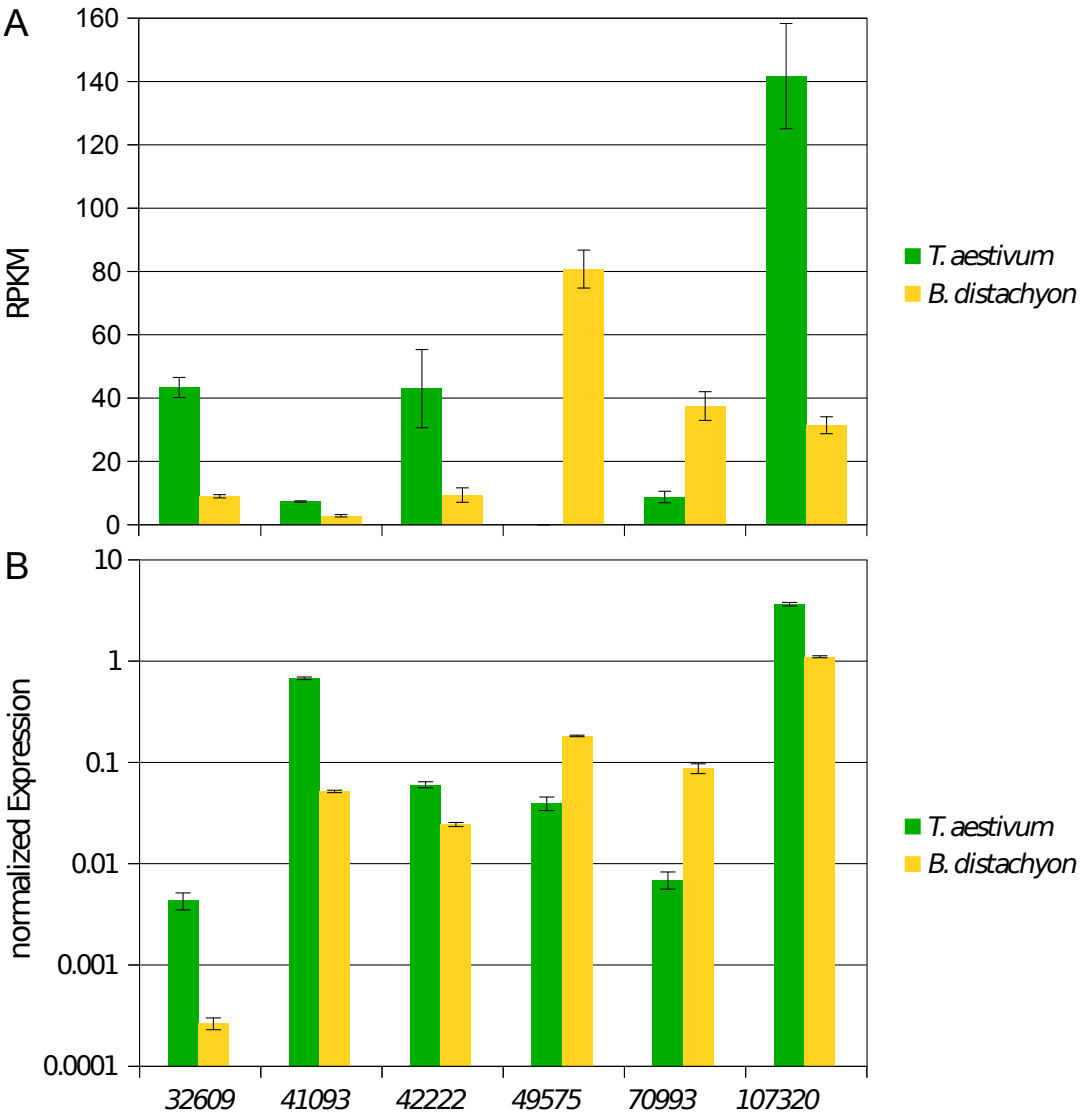


Figure S9 - qRT-PCR validation of RNAseq.
Comparison of six fungal *Z. tritici* genes showing differential expression between infections of *T. aestivum* and *B. distachyon* at 4 days post inoculation. A - Mean RPKM values of replicates from RNAseq analyses. B - Normalized levels of gene expression (against *gapdh*).

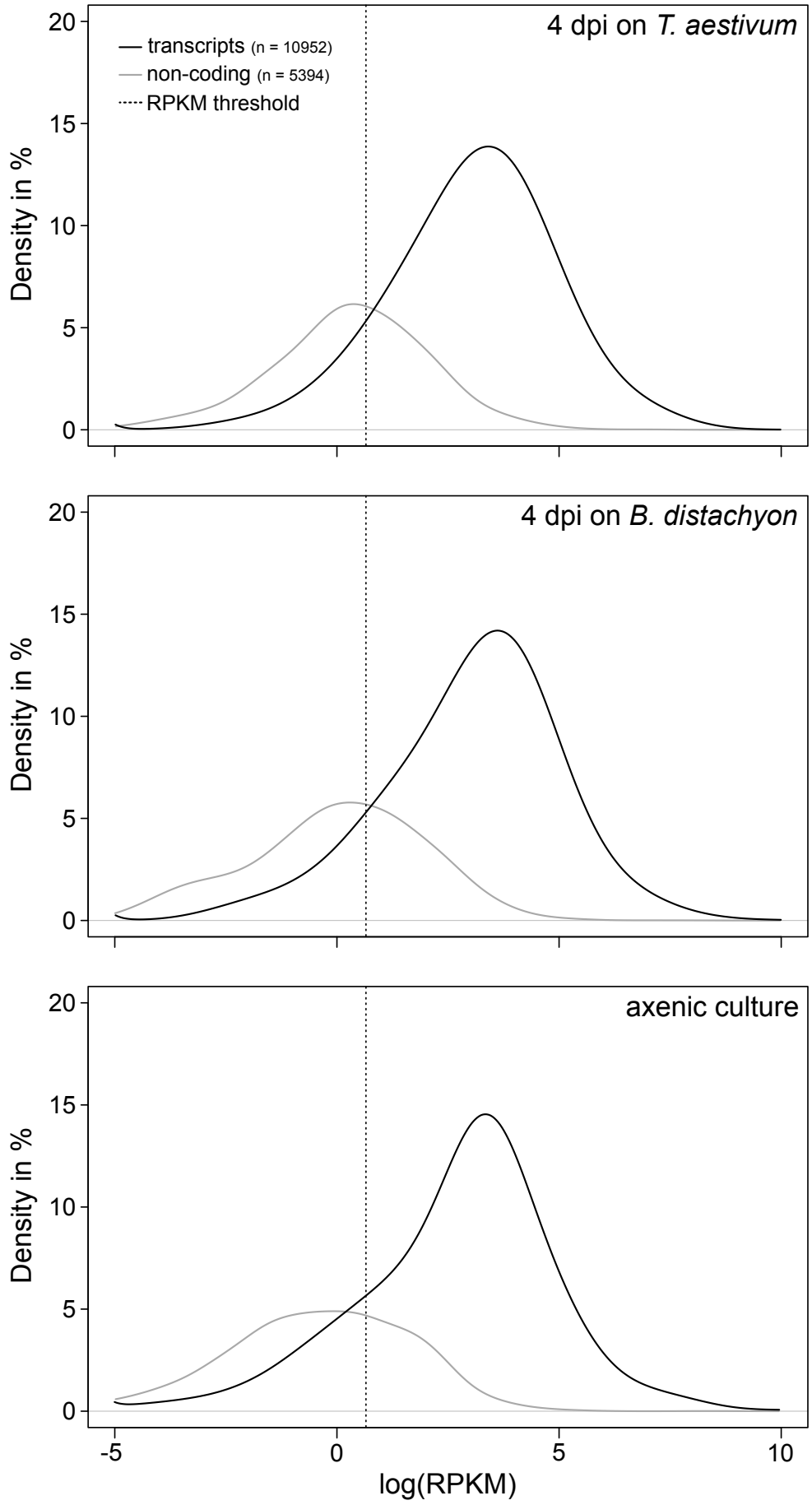


Figure S10 - Assessment of a minimum expression RPKM threshold by comparison of read mapping in coding and non-coding regions. RPKM density plots of 10952 transcripts and 5394 non-coding regions of *Z. tritici*. RPKM values represent mean values of three replicates from axenic culture and 4 days post inoculation (dpi) on *T. aestivum* and *B. distachyon*. Vertical dashed lines indicate the threshold ultimately selected to define expressed loci.